(FILE 'HOME' ENTERED AT 10:56:24 ON 08 NOV 2001)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:57:54 ON 08 NOV 2001

```
L1         60112 S PEG
L2             0 S POLYETHYLENE ADJ GLYCOL
L3        110582 S POLYETHYLENE (A) GLYCOL
L4        146676 S L1 OR L3
L5        885718 S MOLECULAR(W) WEIGHT
L6         40836 S "8000" OR "10000" OR "18000"
L7          5333 S L5 AND L6
L8           576 S L4 AND L7
L9        124212 S COVALENT OR IMMOBLI?
L10       247404 S (SUPEROXIDE (A)DISMUTASE?) OR CATALASE? OR (GLUTATHIONE(A)
PE
L11         1277 S L9 AND L10
L12            0 S L8 AND L11
L13          113 S L11 AND L4
L14           30 S L5 AND L13
L15           16 DUP REM L14 (14 DUPLICATES REMOVED)
L16      2362944 S WOUND OR BANDAGE OR COMPRESS? OR PLASTER? OR SHEET? OR FILM?
L17            0 S L15 AND L16
L18        70993 S ISOCYANATE?
L19            0 S L18 AND L15
L20        48865 S DIISOCYANATE?
L21            0 S L15 AND L20
L22            0 S L15 AND (UREA OR URETHANE?)
L23            6 S L15 AND AMINO
L24            6 DUP REM L23 (0 DUPLICATES REMOVED)
                 E ETTNER N/AU
L25           26 S E3
L26            0 S L25 AND L3
L27            6 S L24 AND L4
L28            6 DUP REM L27 (0 DUPLICATES REMOVED)
L29            0 S L28 AND L6
                 E SCHINK M/AU
L30           34 S E3
L31            0 S L4 AND L30
                 E SCHREIBER J/AU
L32          811 S E3
L33            0 S L32 AND L4
                 E MEIER W/AU
L34         1206 S E3
L35            3 S L4 AND L34
L36            2 DUP REM L35 (1 DUPLICATE REMOVED)
                 E SAUER M/AU
L37          550 S E3
L38            0 S L4 AND L37
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* * * * * * * * * *   Welcome to STN International   * * * * * * * * * *

 NEWS  1           Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2  Dec 17   The CA Lexicon available in the CAPLUS and CA files
 NEWS  3  Feb 06   Engineering Information Encompass files have new names
 NEWS  4  Feb 16   TOXLINE no longer being updated
 NEWS  5  Apr 23   Search Derwent WPINDEX by chemical structure
 NEWS  6  Apr 23   PRE-1967 REFERENCES NOW SEARCHABLE IN CAPLUS AND CA
 NEWS  7  May 07   DGENE Reload
 NEWS  8  Jun 20   Published patent applications (A1) are now in USPATFULL
 NEWS  9  JUL 13   New SDI alert frequency now available in Derwent's
                   DWPI and DPCI
 NEWS 10  Aug 23   In-process records and more frequent updates now in
                   MEDLINE
 NEWS 11  Aug 23   PAGE IMAGES FOR 1947-1966 RECORDS IN CAPLUS AND CA
 NEWS 12  Aug 23   Adis Newsletters (ADISNEWS) now available on STN
 NEWS 13  Sep 17   IMSworld Pharmaceutical Company Directory name change
                   to PHARMASEARCH
 NEWS 14  Oct 09   Korean abstracts now included in Derwent World Patents
                   Index
 NEWS 15  Oct 09   Number of Derwent World Patents Index updates increased
 NEWS 16  Oct 15   Calculated properties now in the REGISTRY/ZREGISTRY File
 NEWS 17  Oct 22   Over 1 million reactions added to CASREACT
 NEWS 18  Oct 22   DGENE GETSIM has been improved
 NEWS 19  Oct 29   AAASD no longer available

 NEWS EXPRESS  August 15 CURRENT WINDOWS VERSION IS V6.0c,
               CURRENT MACINTOSH VERSION IS V6.0 (ENG) AND V6.0J (JP),
               AND CURRENT DISCOVER FILE IS DATED 07 AUGUST 2001
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FILE 'HOME' ENTERED AT 10:56:24 ON 08 NOV 2001

=> file medline embase biosis biotechds scisearch hcaplus ntis lifesci

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                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.45       0.45

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FILE 'LIFESCI' ENTERED AT 10:57:54 ON 08 NOV 2001
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=> s peg

L1       60112 PEG

=> s polyethylene adj glycol

L2           0 POLYETHYLENE ADJ GLYCOL

=> s polyethylene (a) glycol

L3      110582 POLYETHYLENE (A) GLYCOL

=> s l1 or l3

L4      146676 L1 OR L3

=> s molecular(w) weight

L5      885718 MOLECULAR(W) WEIGHT

=> s "8000" or "10000" or "18000"

L6       40836 "8000" OR "10000" OR "18000"

=> s l5 and l6

L7        5333 L5 AND L6

=> s l4 and l7

L8         576 L4 AND L7

=> s covalent or immobli?

L9      124212 COVALENT OR IMMOBLI?

=> s (superoxide (a)dismutase?) or catalase? or (glutathione(a) peroxidase?)
or myeloperoxidase?

   7 FILES SEARCHED...
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L10 247404 (SUPEROXIDE (A) DISMUTASE?) OR CATALASE? OR (GLUTATHIONE(A) PEROXI[redacted]E?) OR MYELOPEROXIDASE?

=> s l9 and l10

L11 1277 L9 AND L10

=> s l8 and l11

L12 0 L8 AND L11

=> s l11 and l4

L13 113 L11 AND L4

=> s l5 and l13

L14 30 L5 AND L13

=> dup rem l14

PROCESSING COMPLETED FOR L14
L15 16 DUP REM L14 (14 DUPLICATES REMOVED)

=> s wound or bandage or compress? or plaster? or sheet? or film?

L16 2362944 WOUND OR BANDAGE OR COMPRESS? OR PLASTER? OR SHEET? OR FILM?

=> s l15 and l16

L17 0 L15 AND L16

=> d l15 1-16 ibib ab

L15 ANSWER 1 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 2001:114920 SCISEARCH
THE GENUINE ARTICLE: 397WZ
TITLE: Peptide and protein PEGylation: a review of problems and solutions
AUTHOR: Veronese F M (Reprint)
CORPORATE SOURCE: Univ Padua, Ctr Chem Invest Drugs, CNR, Dept Pharmaceut Sci, Via F Marzolo 5, I-35131 Padua, Italy (Reprint); Univ Padua, Ctr Chem Invest Drugs, CNR, Dept Pharmaceut Sci, I-35131 Padua, Italy
COUNTRY OF AUTHOR: Italy
SOURCE: BIOMATERIALS, (MAR 2001) Vol. 22, No. 5, pp. 405-417. Publisher: ELSEVIER SCI LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, OXON, ENGLAND. ISSN: 0142-9612.
DOCUMENT TYPE: General Review; Journal
LANGUAGE: English
REFERENCE COUNT: 83
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The paper discusses general problems in using **PEG** for conjugation to high or low **molecular weight** molecules. Methods of binding **PEG** to different functional groups in macromolecules is reported together with their eventual limitations. Problems encountered in conjugation. such as the evaluation of the number of **PEG** chains bound, the localisation of the site of conjugation in polypeptides and the procedure to direct PEGylation to the desired site in the molecule are discussed. Finally, the paper reports on more specific

methods regarding reversible PEGylation, cross-linking reagents with **PEG** arms. **PEG** Fo[redacted]nzyme solubilization in organi[redacted] solvent and new polymers as alternative to **PEG**. (C) 2001 Elsevier Science Ltd. All rights reserved.

L15 ANSWER 2 OF 16 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 1999440547 MEDLINE
DOCUMENT NUMBER: 99440547 PubMed ID: 10510847
TITLE: Bioconjugation in pharmaceutical chemistry.
AUTHOR: Veronese F M; Morpurgo M
CORPORATE SOURCE: Department of Pharmaceutical Sciences, University of Padua,
Italy.. veronese@pdfar3.dsfarm.unipd.it
SOURCE: FARMACO, (1999 Aug 30) 54 (8) 497-516. Ref: 149
Journal code: ACZ; 8912641. ISSN: 0014-827X.
PUB. COUNTRY: Italy
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 20000111
Last Updated on STN: 20000111
Entered Medline: 19991025

AB Polymer conjugation is of increasing interest in pharmaceutical chemistry for delivering drugs of simple structure or complex compounds such peptides, enzymes and oligonucleotides. For long time drugs, mainly with antitumoral activity, have been coupled to natural or synthetic polymers with the purpose of increasing their blood permanence time, taking advantage of the increased mass that reduces kidney ultrafiltration. However only recently complex constructs were devised that exploit the 'enhanced permeability and retention' (EPR) effect for an efficient tumor targeting, the high **molecular weight** for adsorption or receptor mediated endocytosis and finally a lysosomotropic targeting, taking advantage of acid labile bonds or cathepsin susceptible polypeptide spacers between polymer and drug. New original, very active conjugates of this type, as those based on poly(hydroxyacrylate) polymers, are already in advanced state of development. Labile oligonucleotides, including antisense drugs, were also successfully coupled to polymers in view of an increased cell penetration and stabilization towards nucleases. However, the most active research activity resides in the field of polypeptides and proteins delivery, mainly for the two following reasons: first of all because a great number of therapeutically interesting compounds are now being produced by genetic engineering in large quantity and, secondly, because these products are difficult to administer to patients for several inherent drawbacks. Proteins are in fact easily digested by many endo- and exo-peptidases present in blood or in other body districts; most of them are immunogenic to some extent and, finally, they are rapidly excreted by kidney ultrafiltration. **Covalent** polymer conjugation at protein surface was demonstrated to reduce or eliminate these problems, since the bound polymer behaves like a shield hindering the approach of proteolytic enzymes, antibodies, or antigen processing cell. Furthermore, the increase of the **molecular weight** of the conjugate allows to overcome the kidney elimination threshold. Many successful results were already obtained in peptides and proteins, conjugated mainly to water soluble or amphiphilic polymers like poly(ethylene glycol) (**PEG**), dextrans, or styrenemaleic acid anhydride. Among the most successful are the conjugates of asparaginase, interleukin-2 or -6 and neocarcinostatin, to remind some antitumor agents, adenosine deaminase employed in a genetic desease treatment, **superoxide**

dismutase as scavenger of toxic radicals, hemoglobin as oxygen carrier and urok[redacted]se and streptokinase as protei[redacted]with antithrombotic activity. In pharmaceutical chemistry the conjugation with polymers is also of great importance for synthetic applications since many enzymes without loss of catalytic activity become soluble in organic solvents where many drug precursors are. The various and often difficult chemical problems encountered in conjugation of so many different products prompted the development of many synthetic procedures, all characterized by high specificity and mild condition of reaction, now known as 'bioconjugation chemistry'. Bioconjugation developed also the design of new tailor-made polymers with the wanted **molecular weight**, shape, structure and with the functional groups needed for coupling at the wanted positions in the chain.

L15 ANSWER 3 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 97:286673 SCISEARCH
THE GENUINE ARTICLE: WR444
TITLE: Prolongation of the serum half-life period of **superoxide dismutase** by poly(ethylene glycol) modification
AUTHOR: Nakaoka R; Tabata Y; Yamaoka T; Ikada Y (Reprint)
CORPORATE SOURCE: KYOTO UNIV, BIOMED ENGN RES CTR, SAKYO KU, 53 KAWAHARA CHO, KYOTO 60601, JAPAN (Reprint); KYOTO UNIV, BIOMED ENGN RES CTR, SAKYO KU, KYOTO 60601, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: JOURNAL OF CONTROLLED RELEASE, (2 JUN 1997) Vol. 46, No. 3, pp. 253-261. Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 0168-3659.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 33

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Superoxide dismutase** (SOD) was chemically modified using poly(ethylene glycol) (**PEG**) with different molecular weights to prepare **PEG**-SOD conjugates with different extents of modification. The body distribution of the conjugates intravenously injected to mice was investigated to assess the influence of modification on the serum half-life period of SOD. The SOD modification with **PEG** was effective in lowering the elimination rate of SOD from the blood circulation without any change in the distribution pattern of organs other than the kidney. The **molecular weight** of **PEG** used for modification and the modification extent have a minimum effect on the half-life of the SOD. The half-life of the SOD and its **PEG** conjugates have a similar dependency on the apparent **molecular weight** as the **PEG** molecules. This indicates that the half-life of SOD and the **PEG** conjugates are mainly determined by their molecular size.

L15 ANSWER 4 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 1998:12669 SCISEARCH
THE GENUINE ARTICLE: BK03M
TITLE: Conjugation of high-**molecular weight** poly(ethylene glycol) to cytokines: Granulocyte-macrophage colony-stimulating factors as model substrates
AUTHOR: Sherman M R (Reprint); Williams L D; Saifer M G P; French J A; Kwak L W; Oppenheim J J
CORPORATE SOURCE: MT VIEW PHARMACEUT INC, 871-L IND PK, MENLO PK, CA 94025 (Reprint); NCI, FREDERICK CANC RES & DEV CTR, FREDERICK,

MD 21702
COUNTRY OF AUTHOR:
SOURCE: ACS SYMPOSIUM SERIES, (FEB 1997) Vol. 680, pp. 155-169.
Publisher: AMER CHEMICAL SOC, 1155 SIXTEENTH ST NW, WASHINGTON, DC 20036.
ISSN: 0097-6156.
DOCUMENT TYPE: General Review; Journal
LANGUAGE: English
REFERENCE COUNT: 71

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The ability of the small receptor-binding protein, recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF), to increase the abundance of certain blood cell types in mice was enhanced markedly by **covalent** attachment of a single long strand of **PEG** (30-40 kDa). Potency was not increased further by coupling a second strand. Such conjugates can be synthesized efficiently by reaction of protein amino groups with **PEG** propionaldehydes in the presence of NaBH3CN or with **PEG** p-nitrophenyl carbonates. Both methods have been used to prepare recombinant human GM-CSF conjugates of predetermined composition, e.g. **PEG**(1)GM-CSF and **PEG**(2)GM-CSF, in high yield. These compounds, or analogous derivatives of other cytokines, purified by ion-exchange and size-exclusion chromatography, may be suitable candidates for pharmaceutical development.

L15 ANSWER 5 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 97:222875 SCISEARCH
THE GENUINE ARTICLE: WN017
TITLE: A simple and efficient method for preparation of monomethoxypolyethylene glycol activated with p-nitrophenylchloroformate and its application to modification of L-asparaginase
AUTHOR: Kito M; Miron T; Wilchek M; Kojima N; Ohishi N; Yagi K (Reprint)
CORPORATE SOURCE: INST APPL BIOCHEM, YAGI MEM PK, GIFU 50501, JAPAN (Reprint); INST APPL BIOCHEM, GIFU 50501, JAPAN; WEIZMANN INST SCI, DEPT BIOPHYS, IL-76100 REHOVOT, ISRAEL
COUNTRY OF AUTHOR: JAPAN; ISRAEL
SOURCE: JOURNAL OF CLINICAL BIOCHEMISTRY AND NUTRITION, (SEP 1996)
Vol. 21, No. 2, pp. 101-111.
Publisher: INST APPLIED BIOCHEMISTRY, YAGI MEMORIAL PARK, MITAKE GIFU 505-01, JAPAN.
ISSN: 0912-0009.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 28

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB An improved, simple and efficient method for preparation of monomethoxypolyethylene glycol (**PEG**) activated with p-nitrophenylchloroformate (PNP-**PEG**) and its use as a potent modifier of protein under mild conditions are described. Modification of bovine serum albumin with PNP-**PEG** was compared with that done with **PEG** activated with N,N'-carbonyldiimidazole or cyanuric chloride. The reaction of **PEG**, activated with either p-nitrophenylchloroformate or cyanuric chloride, with bovine serum albumin at 4 degrees C reached a plateau within 1 h, whereas protein modification using **PEG** activated,with N,N'-carbonyldiimidazole was rather slow and gave a low yield. The remaining activity of L-asparaginase modified with PNP-**PEG** was much higher than that of the enzyme modified to the same degree with **PEG** activated with cyanuric chloride. At a 20 molar excess of PNP-**PEG** having a **molecular weight** of 5,000, 55% of the free amino acid

groups were modified at 4 degrees C for 2 h, and the modified enzyme still had 33% residual enzyme activity. Immunochemical studies showed that the highly modified enzyme (67% modification with 18% residual enzyme activity) had lost its immunogenicity and had become much less sensitive to protease digestion.

L15 ANSWER 6 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 96:257769 SCISEARCH
THE GENUINE ARTICLE: UB814
TITLE: SYNTHESIS, CHARACTERIZATION AND PROPERTIES OF SIALYLATED **CATALASE**
AUTHOR: FERNANDES A I; GREGORIADIS G (Reprint)
CORPORATE SOURCE: UNIV LONDON, SCH PHARM, CTR DRUG DELIVERY RES, 29-39 BRUNSWICK SQ, LONDON WC1N 1AX, ENGLAND (Reprint); UNIV LONDON, SCH PHARM, CTR DRUG DELIVERY RES, LONDON WC1N 1AX, ENGLAND
COUNTRY OF AUTHOR: ENGLAND
SOURCE: BIOCHIMICA ET BIOPHYSICA ACTA-PROTEIN STRUCTURE AND MOLECULAR ENZYMOLOGY, (07 MAR 1996) Vol. 1293, No. 1, pp. 90-96.
ISSN: 0167-4838.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 35

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Colominic acid (CA), a alpha-(2 --> 8) N-acetylneuraminic acid (sialic acid) polymer (average **molecular weight** of 10 kDa) was activated by periodate oxidation of carbon 7 at the non-reducing end of the saccharide. The oxidized CA was then coupled to **catalase** by reductive amination in the presence of sodium cyanoborohydride. The extent of sialylation of **catalase**, estimated by ammonium sulfate precipitation as 3.8 +/- 0.4 (mean +/- S.D.) moles of CA per mole of **catalase**, did not improve significantly when depolymerized CA was used in the coupling reaction. At the end of the coupling reaction, sialylated **catalase** exhibited a two-fold (70%) retention of initial activity compared to enzyme controls (29-35%) subjected to the same conditions. Formation of sialylated **catalase** was confirmed by ammonium sulfate or trichloroacetic acid precipitation, molecular sieve chromatography and SDS-PAGE electrophoresis. Enzyme kinetics studies revealed an increase in the apparent K-m of the enzyme from 70.0 (native) to 122.9 mmol l(-1) H2O2 (sialylated **catalase**) indicating a reduction of enzyme affinity for the substrate (hydrogen peroxide) on sialylation. Compared to native enzyme, sialylated **catalase** was much more stable in the presence of specific proteinases, completely resisting degradation by chymotrypsin and losing only some of its activity in the presence of trypsin. The increased stability conferred to **catalase** by sialylation agrees with similar observations on enzymes modified by other hydrophilic molecules (e.g., monomethoxypoly(ethyleneglycol)) and suggests that steric stabilization with the biodegradable polysialic acid may prove an alternative means to render therapeutic proteins more effective in vivo.

L15 ANSWER 7 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 95:443915 SCISEARCH
THE GENUINE ARTICLE: RE939
TITLE: COMPARISON OF BODY DISTRIBUTION OF POLY(VINYL ALCOHOL) WITH OTHER WATER-SOLUBLE POLYMERS AFTER INTRAVENOUS ADMINISTRATION
AUTHOR: YAMAOKA T; TABATA Y; IKADA Y (Reprint)
CORPORATE SOURCE: KYOTO UNIV, BIOMED ENGN RES CTR, SAKYO KU, 53 KAWAHARA

CHO, KYOTO 606, JAPAN (Reprint); KYOTO UNIV, BIOMED ENGN [redacted] CTR, SAKYO KU, KYOTO 606, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: JOURNAL OF PHARMACY AND PHARMACOLOGY, (JUN 1995) Vol. 47, No. 6, pp. 479-486.
ISSN: 0022-3573.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 36

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The body distribution of poly(vinyl alcohol) (PVA) with molecular weights (MW) from 14800 to 434000 Da was investigated after intravenous administration and compared with that of other water-soluble polymers such as poly(ethylene glycol) (**PEG**), gelatin, dextran, and pullulan.

The half-life of PVA in the circulation was prolonged from 90 min (MW 14800Da) to 23 h (MW 434000 Da), similar to that of **PEG** which had a half-life of 30 min (MW 6000) and 20 h (MW 170000). However, the half-life of PVA was much longer than that of other polymers when compared at a similar **molecular weight**. PVA was located in most organs but with very small accumulation. An insignificant interaction of PVA with cell components, such as macrophages and blood cells, was observed. Similar to **PEG**, the excretion rate of PVA at the glomeruli was rapidly reduced around 30000 Da, as the **molecular weight** increased.

These results indicate that the half-life of intravenously injected PVA in the blood was mainly determined by the permeation characteristics of the kidney.

L15 ANSWER 8 OF 16 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:213658 HCAPLUS
DOCUMENT NUMBER: 124:279129
TITLE: The effect of human recombinant **superoxide dismutase** conjugated with **polyethylene glycol** on the hepatic toxicity of acetaminophen
AUTHOR(S): Yong, Chul Soon; Park, Kyong-Ah; Oh, Doo-Man
CORPORATE SOURCE: Coll. Pharmacy, Yeungnam Univ., Gyongsan, 712-749, S. Korea
SOURCE: Yakche Hakhoechi (1995), 25(4), 313-22
CODEN: YAHAEX; ISSN: 0259-2347
DOCUMENT TYPE: Journal
LANGUAGE: Korean

AB The **covalent** conjugation of human recombinant **superoxide dismutase** (hrSOD) with trichloro-s-triazine-activated **polyethylene glycol** (PGE) 5000 formed sol. conjugates with **mol. wt.** of 92 kD, which retained 90.apprx.98% of original activity with a markedly prolonged plasma half-life of enzyme activity. The effect of hrSOD-**Peg** conjugates on acetaminophen (ACP)-induced hepatotoxicity was tested in male rats which were pretreated with 3-methylcholanthrene. HrSOD-**PEG** conjugates inhibited the hepatotoxicity produced by ACP; on the other hand, native hrSOD had no protective effect. The above results indicated that oxygen radicals might participate in the mechanism of the ACP-induced hepatotoxicity and that polymer conjugated-protein drugs with prolonged half-lives could be employed as an effective therapeutic agent.

L15 ANSWER 9 OF 16 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 2
ACCESSION NUMBER: 95309405 EMBASE
DOCUMENT NUMBER: 1995309405
TITLE: Chemistry of **polyethylene glycol**

conjugates with biologically.
AUTHOR: Z[redacted]osky S.
CORPORATE SOURCE: SEQUUS Pharmaceuticals Inc, 960 Hamilton Court,Menlo Park, CA 94025, United States
SOURCE: Advanced Drug Delivery Reviews, (1995) 16/2-3 (157-182). ISSN: 0169-409X CODEN: ADDREP
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 027 Biophysics, Bioengineering and Medical Instrumentation
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB **Polyethylene glycol** (**PEG**) is widely used as a **covalent** modifier of biological macromolecules and particulates as we]l as a carrier for low **molecular weight** drugs. In the first two instances proteins and liposomes are of particular importance. Their conjugates with **PEG** often possess the ability to avoid quick recognition and clearance in vivo, that their unconjugated counterparts are suffering from. In this review (with 133 references) methods for preparation of **PEG** conjugates with various biologically active compounds are summarized. Since the bulk of the published work in this field involves proteins, drugs, and lipids. an appropriate emphasis is given to the conjugates of these compounds. While the first two types of **PEG** conjugates are usually intended for a direct use as therapeutics, **PEG**-lipids are mainly utilized for formation of long-circulating liposomes. Particular attention is paid to the comparative attributes of various reactive **PEG** derivatives, properties of the linkages formed, and possible side reactions. The relationships between various conjugation strategies and their influence on the relevant biological properties and/or on in vivo performance of the corresponding conjugates is also discussed.

L15 ANSWER 10 OF 16 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 94:220960 SCISEARCH
THE GENUINE ARTICLE: NF270
TITLE: DISTRIBUTION AND TISSUE UPTAKE OF POLY(ETHYLENE GLYCOL) WITH DIFFERENT MOLECULAR-WEIGHTS AFTER INTRAVENOUS ADMINISTRATION TO MICE
AUTHOR: YAMAOKA T; TABATA Y; IKADA Y (Reprint)
CORPORATE SOURCE: KYOTO UNIV, BIOMED ENGN RES CTR, SAKYO KU, 53 KAWAHARA CHO SHOGOIN, KYOTO 606, JAPAN (Reprint); KYOTO UNIV, BIOMED ENGN RES CTR, SAKYO KU, KYOTO 606, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: JOURNAL OF PHARMACEUTICAL SCIENCES, (APR 1994) Vol. 83, No. 4, pp. 601-606. ISSN: 0022-3549.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 38
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB After intravenous (iv) injection of I-125-labeled poly(ethylene glycol) (**PEG**) with different molecular weights to mice, the radioactivity of the organs was measured to pharmacokinetically analyze the body distribution of **PEG** according to a two-compartment model. High **molecular weight** PEGs were retained in the blood circulation for a longer period than low **molecular weight** PEGs. The terminal half-life of **PEG** in the circulation extended from 18 min to 1 day as the **PEG**

molecular weight increased from 6000 to 190 000. **PEG** tended to accumulate in the tissues/organs such as muscle, skin, bone, and the liver to a higher extent than the other organs, irrespective of the **molecular weight**. The time dependence of tissue accumulation was based on the vascular permeability. The results of pharmacokinetic analysis suggested that small **PEG** tended to freely translocate from the circulation to extravascular tissues and to return to the blood circulation again by diffusion, whereas large **PEG** translocated more slowly to extravascular tissues. Urinary clearance decreased with increasing **PEG molecular weight**, similar to the tissue clearance, whereas liver clearance increased with the increasing **PEG molecular weight**, after passing a minimum around the **molecular weight** of 50 000. **PEG** uptake by Kupffer cells was enhanced as the **molecular weight** became >50 000.

L15 ANSWER 11 OF 16 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 3
ACCESSION NUMBER: 1995:16065 BIOSIS
DOCUMENT NUMBER: PREV199598030365
TITLE: Acylation of amino functions of proteins with monomethoxypoly (ethylene glycol)-N-succinimide carbonate.
AUTHOR(S): Nijs, Michelle; Gelbcke, Michel; Azarkan, Mohamed; Brygier, Jeanne; Guermant, Claude; Baeyens-Volant, Danielle; Musu, Tony; Paul, Claudine; Looze, Yvan (1)
CORPORATE SOURCE: (1) Protein Chem. Unit, Fac. Med., Univ. Brussels, Brussels Belgium
SOURCE: Applied Biochemistry and Biotechnology, (1994) Vol. 49, No. 1, pp. 75-91.
ISSN: 0273-2289.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Monomethoxypoly(ethylene glycol)-N-succinimide carbonate (SC-**PEG**) was used to prepare **PEG**-lysozyme, **PEG**-papaya proteinase 111, **PEG-catalase**, and **PEG**-lactoperoxidase conjugates. SC-**PEG** produced extensively modified enzymes under mild conditions (pH 7.0; 25 degree C) within a couple of hours. **PEG**-enzyme conjugates showed equal or even greater specific activity provided that low-**molecular-weight** substrates were used to evaluate the biological activities. However, papaya proteinase III and lysozyme lost their proteolytic and bacteriolytic activities, respectively, on conjugation with **PEG**. This was most probably because of steric factors, since no drastic conformational changes could be detected after conjugation of these enzymes with **PEG** chains. Unlike these enzymes, the secondary structures of the two hemoproteins were somewhat affected by the **covalent** attachment of **PEG** chains as shown by FTIR experiments. These results confirmed the potential usefulness of SC-**PEG**, for which a novel route of synthesis making use of N,N'-disuccinimidyl carbonate was described.

L15 ANSWER 12 OF 16 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 93289947 MEDLINE
DOCUMENT NUMBER: 93289947 PubMed ID: 8512060
TITLE: Reagents for the preparation of chromophorically labeled **polyethylene glycol**-protein conjugates.
AUTHOR: Ladd D L; Snow R A
CORPORATE SOURCE: Medicinal Chemistry Department, Sterling Winthrop Pharmaceuticals Research Division, Sterling Winthrop Inc., Malvern, Pennsylvania 19355.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1993 May 1) 210 (2) 258-61.
Journal code: 4NK; 0370535. ISSN: 0003-2697.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19930723
Last Updated on STN: 19930723
Entered Medline: 19930709

AB We have developed a new class of reagents (2) for the **covalent** attachment of **polyethylene glycol** to proteins. These reagents (2) are the monomethoxypolyethylene glycol esters of 4-fluoro-3-nitrobenzoic acid. The reaction of 2 with lysine epsilon-amino groups produces a chromophore which can be used to quantitate the **polyethylene glycol** to protein molar ratio. Bovine (Zn, Cu) **superoxide dismutase** was used as a model protein for conjugation with 2. When monomethoxypolyethylene glycol of average **molecular weight** 2105 was used, a conjugate was obtained with a **polyethylene glycol** to protein molar ratio of 8.88 retaining 100% of native enzymatic activity; monomethoxypolyethylene glycol of average **molecular weight** 5210 yielded a conjugate with a **polyethylene glycol** to protein molar ratio of 9.96 retaining 73% of native enzymatic activity.

L15 ANSWER 13 OF 16 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 90339014 MEDLINE
DOCUMENT NUMBER: 90339014 PubMed ID: 2166134
TITLE: Spectroscopic characterization of polyethyleneglycol modified **superoxide dismutase**: 1H NMR studies on its Cu2Co2 derivative.
AUTHOR: Banci L; Bertini I; Caliceti P; Monsu Scolaro L; Schiavon O; Veronese F M
CORPORATE SOURCE: Department of Chemistry, University of Florence, Italy.
SOURCE: JOURNAL OF INORGANIC BIOCHEMISTRY, (1990 Jun) 39 (2) 149-59.
Journal code: JAR; 7905788. ISSN: 0162-0134.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199009
ENTRY DATE: Entered STN: 19901012
Last Updated on STN: 19901012
Entered Medline: 19900910

AB Spectroscopic methods have been employed in order to understand the molecular basis of the decrease in enzymatic activity of the antiinflammatory enzyme copper-zinc **superoxide dismutase** (SOD) following the **covalent** binding of polyethyleneglycol (**PEG**) chains to the protein amino-groups. The **PEG** modification is a general method recently proposed to improve the therapeutic index of enzymes. 1H NMR spectra on the cobalt substituted **PEG**-modified SOD, Cu2Co2-**PEG**-SOD, have been recorded. The signals are quite broad with respect to the unmodified enzyme. This has been interpreted on the basis of the effect of **molecular weight** on the linewidth. The analysis has shown that the histidine hydrogens involved in metal binding at the enzyme active site are the same in both native and **PEG**-modified SOD. Similarly, circular dichroism and absorption spectra indicate that the overall conformation of the metal clusters is not perturbed upon modification. On the other hand, azide titration shows that the affinity constant of N-3 for SOD is largely reduced upon **PEG** modification (K = 154 M-1 and 75 M-1 for the native and modified SOD, respectively). These results indicate that the decrease in enzymatic activity upon surface modification with **PEG** is not caused by a perturbation of the active site geometry, but to a

decrease in the channeling of the O2- ion towards the enzyme active site.

L15 ANSWER 14 OF 16 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 6
ACCESSION NUMBER: 1988:507428 BIOSIS
DOCUMENT NUMBER: BA86:128112
TITLE: ANALYSIS OF **POLYETHYLENE GLYCOL** MODIFIED **SUPEROXIDE DISMUTASE** BY CHROMATOGRAPHIC ELECTROPHORETIC LIGHT SCATTERING CHEMICAL AND ENZYMATIC METHODS.
AUTHOR(S): MCGOFF P; BAZIOTIS A C; MASKIEWICZ R
CORPORATE SOURCE: BOEHRINGER INGELLHEIM PHARMACEUTICALS INC., RIDGEFIELD, CONN. 06877, USA.
SOURCE: CHEM PHARM BULL (TOKYO), (1988) 36 (8), 3079-3091. CODEN: CPBTAL. ISSN: 0009-2363.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB **Covalent** conjugation of bovine erythrocyte **superoxide dismutase** (SOD) with activated **polyethylene glycol** (**PEG**) results in a mixture of modified species (**PEG**-SOD) with properties different from those of the native enzyme. The components of this mixture were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), isoelectric focusing, and chromatographic (size-exclusion, anion-exchange, cation-exchange and reverse-phase high performance liquid chromatography) techniques. Physicochemical properties such as apparent **molecular weight**, isoelectric point, relative hydrophobicity and relative cation-anion charge number were measured by electrophoretic and chromatographic procedures. Dispersity and apparent radius were examined by chromatographic and light scattering techniques. The extent of **covalent** modification and enzymatic activity change were measured by chemical and spectroscopic methods, showing that activity loss was not due to catalytic site modification. The properties of the **PEG**-modified form of the enzyme were compared with those of native SOD and showed that in addition to changing biological properties, **PEG** modification of proteins can result in a product with unexpectedly high heterogeneity and substantial changes in isoelectric point and hydrophobicity. Altered biological properties may therefore not merely be due to shielding of protein surface by **PEG** chains. Apparent properties of **PEG** modified proteins such as **molecular weight** were found to be highly method dependent, with poor agreement being shown among classical measurements.

L15 ANSWER 15 OF 16 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1979:451927 HCAPLUS
DOCUMENT NUMBER: 91:51927
TITLE: Preparation and properties of **polyethylene glycol**-trypsin adducts
AUTHOR(S): Abuchowski, Abraham; Davis, Frank F.
CORPORATE SOURCE: Bur. Biol. Res., Rutgers, State Univ., New Brunswick, NJ, 08903, USA
SOURCE: Biochim. Biophys. Acta (1979), 578(1), 41-6 CODEN: BBACAQ; ISSN: 0006-3002
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The **covalent** attachment of **polyethylene glycol** (**mol. wt.**, 5000 daltons) to nonessential groups on trypsin produced an adduct that no longer pptd. with antitrypsin antibody.
In comparison with trypsin, **polyethylene glycol**-trypsin prepns. showed equal or greater activity against N-.alpha.-benzoyl-L-arginine Et ester, .apprx.1/4 activity against angiotensin II, and little activity against bovine liver **catalase**. The adduct dissolved soft blood clots at 1/4 the rate of trypsin. Soybean trypsin inhibitor produced 2/3 inhibition of the adduct under conditions that caused complete inhibition of trypsin.

L15 ANSWER 16 OF 16 MEDLINE
ACCESSION NUMBER: [redacted]7848 MEDLINE
DOCUMENT NUMBER: 77187848 PubMed ID: 16907
TITLE: Effect of **covalent** attachment of **polyethylene glycol** on immunogenicity and circulating life of bovine liver **catalase**.
AUTHOR: Abuchowski A; McCoy J R; Palczuk N C; van Es T; Davis F F
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1977 Jun 10) 252 (11) 3582-6.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197707
ENTRY DATE: Entered STN: 19900314
Last Updated on STN: 19950206
Entered Medline: 19770723

AB Methoxypolyethylene glycols of 1900 daltons (**PEG**-1900) or 5000 daltons (**PEG**-5000) were covalently attached to bovine liver **catalase** using 2,4,6-trichloro-s-triazine as the coupling agent. Rabbits were immunized by the intravenous and intramuscular routes with **catalase** modified by **covalent** attachment of **PEG** -1900 to 43% of the amino groups (**PEG**-1900-**catalase**). The intravenous antiserum did not yield detectable antibodies against **PEG**-1900-**catalase** or native **catalase**, as determined by Ouchterlony and complement fixation methods, whereas the intramuscular antiserum contained antibodies to both **PEG**-1900-**catalase** and **catalase**. **PEG**-1900 did not react with either antiserum. **Catalase** was prepared in which **PEG**-5000 was attached to 40% of the amino groups (**PEG** -5000-**catalase**). This **catalase** preparation did not react with either antiserum. **PEG**-1900-**catalase** retained 93% of its enzymatic activity; **PEG**-5000-**catalase** retained 95%. **PEG**-5000-**catalase** resisted digestion by trypsin, chymotrypsin, and a protease from Streptomyces griseus. **PEG**-1900-**catalase** and **PEG**-5000-**catalase** exhibited enhanced circulating lives in the blood of acatalasemic mice during repetitive intravenous injections. No evidence was seen of an immune response to injections of the modified enzymes. Mice injected repetitively with **PEG**-5000-**catalase** remained immune competent for unmodieied **catalase** , and no evidence of tissue or organ damage was seen.

=> d his

(FILE 'HOME' ENTERED AT 10:56:24 ON 08 NOV 2001)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:57:54 ON 08 NOV 2001

```
L1        60112 S PEG
L2            0 S POLYETHYLENE ADJ GLYCOL
L3       110582 S POLYETHYLENE (A) GLYCOL
L4       146676 S L1 OR L3
L5       885718 S MOLECULAR(W) WEIGHT
L6        40836 S "8000" OR "10000" OR "18000"
L7         5333 S L5 AND L6
L8          576 S L4 AND L7
L9       124212 S COVALENT OR IMMOBLI?
L10      247404 S (SUPEROXIDE (A)DISMUTASE?) OR CATALASE? OR (GLUTATHIONE(A)
PE
L11        1277 S L9 AND L10
L12           0 S L8 AND L11
L13         113 S L11 AND L4
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```
L14          30 S L5 AND L13
L15          16 DUP REM L14 (14 DUPLICATES REMOVED)
L16     2362944 S WOUND OR BANDAGE OR COMPRESS? OR PLASTER? OR SHEET? OR FILM?
L17           0 S L15 AND L16

=> s isocyanate?

L18       70993 ISOCYANATE?

=> s l18 and l15

L19           0 L18 AND L15

=> s diisocyanate?

L20       48865 DIISOCYANATE?

=> s l15 and l20

L21           0 L15 AND L20

=> d his


     (FILE 'HOME' ENTERED AT 10:56:24 ON 08 NOV 2001)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 10:57:54 ON 08 NOV 2001
L1         60112 S PEG
L2             0 S POLYETHYLENE ADJ GLYCOL
L3        110582 S POLYETHYLENE (A) GLYCOL
L4        146676 S L1 OR L3
L5        885718 S MOLECULAR(W) WEIGHT
L6         40836 S "8000" OR "10000" OR "18000"
L7          5333 S L5 AND L6
L8           576 S L4 AND L7
L9        124212 S COVALENT OR IMMOBLI?
L10       247404 S (SUPEROXIDE (A)DISMUTASE?) OR CATALASE? OR (GLUTATHIONE(A)
PE
L11         1277 S L9 AND L10
L12            0 S L8 AND L11
L13          113 S L11 AND L4
L14           30 S L5 AND L13
L15           16 DUP REM L14 (14 DUPLICATES REMOVED)
L16      2362944 S WOUND OR BANDAGE OR COMPRESS? OR PLASTER? OR SHEET? OR FILM?
L17            0 S L15 AND L16
L18        70993 S ISOCYANATE?
L19            0 S L18 AND L15
L20        48865 S DIISOCYANATE?
L21            0 S L15 AND L20

=> s l15 and (urea or urethane?)

L22           0 L15 AND (UREA OR URETHANE?)

=> s l15 and amino

L23           6 L15 AND AMINO

=> dup rem l23

PROCESSING COMPLETED FOR L23
L24           6 DUP REM L23 (0 DUPLICATES REMOVED)

=> d 1-6 ibib ab
```

L24 ANSWER 1 OF 6 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 1998:12669 SCISEARCH
THE GENUINE ARTICLE: BK03M
TITLE: Conjugation of high-**molecular weight** poly(ethylene glycol) to cytokines: Granulocyte-macrophage colony-stimulating factors as model substrates
AUTHOR: Sherman M R (Reprint); Williams L D; Saifer M G P; French J A; Kwak L W; Oppenheim J J
CORPORATE SOURCE: MT VIEW PHARMACEUT INC, 871-L IND PK, MENLO PK, CA 94025 (Reprint); NCI, FREDERICK CANC RES & DEV CTR, FREDERICK, MD 21702
COUNTRY OF AUTHOR: USA
SOURCE: ACS SYMPOSIUM SERIES, (FEB 1997) Vol. 680, pp. 155-169. Publisher: AMER CHEMICAL SOC, 1155 SIXTEENTH ST NW, WASHINGTON, DC 20036. ISSN: 0097-6156.
DOCUMENT TYPE: General Review; Journal
LANGUAGE: English
REFERENCE COUNT: 71
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The ability of the small receptor-binding protein, recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF), to increase the abundance of certain blood cell types in mice was enhanced markedly by **covalent** attachment of a single long strand of **PEG** (30-40 kDa). Potency was not increased further by coupling a second strand. Such conjugates can be synthesized efficiently by reaction of protein **amino** groups with **PEG** propionaldehydes in the presence of NaBH3CN or with **PEG** p-nitrophenyl carbonates. Both methods have been used to prepare recombinant human GM-CSF conjugates of predetermined composition, e.g. **PEG**(1)GM-CSF and **PEG**(2)GM-CSF, in high yield. These compounds, or analogous derivatives of other cytokines, purified by ion-exchange and size-exclusion chromatography, may be suitable candidates for pharmaceutical development.

L24 ANSWER 2 OF 6 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 97:222875 SCISEARCH
THE GENUINE ARTICLE: WN017
TITLE: A simple and efficient method for preparation of monomethoxypolyethylene glycol activated with p-nitrophenylchloroformate and its application to modification of L-asparaginase
AUTHOR: Kito M; Miron T; Wilchek M; Kojima N; Ohishi N; Yagi K (Reprint)
CORPORATE SOURCE: INST APPL BIOCHEM, YAGI MEM PK, GIFU 50501, JAPAN (Reprint); INST APPL BIOCHEM, GIFU 50501, JAPAN; WEIZMANN INST SCI, DEPT BIOPHYS, IL-76100 REHOVOT, ISRAEL
COUNTRY OF AUTHOR: JAPAN; ISRAEL
SOURCE: JOURNAL OF CLINICAL BIOCHEMISTRY AND NUTRITION, (SEP 1996) Vol. 21, No. 2, pp. 101-111. Publisher: INST APPLIED BIOCHEMISTRY, YAGI MEMORIAL PARK, MITAKE GIFU 505-01, JAPAN. ISSN: 0912-0009.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 28
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB An improved, simple and efficient method for preparation of monomethoxypolyethylene glycol (**PEG**) activated with p-nitrophenylchloroformate (PNP-**PEG**) and its use as a potent modifier of protein under mild conditions are described. Modification of

bovine serum albumin with PNP-**PEG** was compared with that done with **PEG** activated with N,N'-carbonyldiimidazole or cyanuric chloride. The reaction of **PEG**, activated with either p-nitrophenylchloroformate or cyanuric chloride, with bovine serum albumin at 4 degrees C reached a plateau within 1 h, whereas protein modification using **PEG** activated,with N,N'-carbonyldiimidazole was rather slow and gave a low yield. The remaining activity of L-asparaginase modified with PNP-**PEG** was much higher than that of the enzyme modified to the same degree with **PEG** activated with cyanuric chloride. At a 20 molar excess of PNP-**PEG** having a **molecular weight** of 5,000, 55% of the free **amino** acid groups were modified at 4 degrees C for 2 h, and the modified enzyme still had 33% residual enzyme activity. Immunochemical studies showed that the highly modified enzyme (67% modification with 18% residual enzyme activity) had lost its immunogenicity and had become much less sensitive to protease digestion.

L24 ANSWER 3 OF 6 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1995:16065 BIOSIS
DOCUMENT NUMBER: PREV199598030365
TITLE: Acylation of **amino** functions of proteins with monomethoxypoly (ethylene glycol)-N-succinimide carbonate.
AUTHOR(S): Nijs, Michelle; Gelbcke, Michel; Azarkan, Mohamed; Brygier, Jeanne; Guermant, Claude; Baeyens-Volant, Danielle; Musu, Tony; Paul, Claudine; Looze, Yvan (1)
CORPORATE SOURCE: (1) Protein Chem. Unit, Fac. Med., Univ. Brussels, Brussels Belgium
SOURCE: Applied Biochemistry and Biotechnology, (1994) Vol. 49, No. 1, pp. 75-91.
ISSN: 0273-2289.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Monomethoxypoly(ethylene glycol)-N-succinimide carbonate (SC-**PEG**) was used to prepare **PEG**-lysozyme, **PEG**-papaya proteinase 111, **PEG-catalase**, and **PEG**-lactoperoxidase conjugates. SC-**PEG** produced extensively modified enzymes under mild conditions (pH 7.0; 25 degree C) within a couple of hours. **PEG**-enzyme conjugates showed equal or even greater specific activity provided that low-**molecular-weight** substrates were used to evaluate the biological activities. However, papaya proteinase III and lysozyme lost their proteolytic and bacteriolytic activities, respectively, on conjugation with **PEG**. This was most probably because of steric factors, since no drastic conformational changes could be detected after conjugation of these enzymes with **PEG** chains. Unlike these enzymes, the secondary structures of the two hemoproteins were somewhat affected by the **covalent** attachment of **PEG** chains as shown by FTIR experiments. These results confirmed the potential usefulness of SC-**PEG**, for which a novel route of synthesis making use of N,N'-disuccinimidyl carbonate was described.

L24 ANSWER 4 OF 6 MEDLINE
ACCESSION NUMBER: 93289947 MEDLINE
DOCUMENT NUMBER: 93289947 PubMed ID: 8512060
TITLE: Reagents for the preparation of chromophorically labeled **polyethylene glycol**-protein conjugates.
AUTHOR: Ladd D L; Snow R A
CORPORATE SOURCE: Medicinal Chemistry Department, Sterling Winthrop Pharmaceuticals Research Division, Sterling Winthrop Inc., Malvern, Pennsylvania 19355.

SOURCE: ANALYTICAL BIOCHEMISTRY, (1993 May 1) 210 (2) 258-61.
Journal code: 4NK; 0370535. ISSN: [redacted]3-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19930723
Last Updated on STN: 19930723
Entered Medline: 19930709

AB We have developed a new class of reagents (2) for the **covalent** attachment of **polyethylene glycol** to proteins. These reagents (2) are the monomethoxypolyethylene glycol esters of 4-fluoro-3-nitrobenzoic acid. The reaction of 2 with lysine epsilon-**amino** groups produces a chromophore which can be used to quantitate the **polyethylene glycol** to protein molar ratio. Bovine (Zn, Cu) **superoxide dismutase** was used as a model protein for conjugation with 2. When monomethoxypolyethylene glycol of average **molecular weight** 2105 was used, a conjugate was obtained with a **polyethylene glycol** to protein molar ratio of 8.88 retaining 100% of native enzymatic activity; monomethoxypolyethylene glycol of average **molecular weight** 5210 yielded a conjugate with a **polyethylene glycol** to protein molar ratio of 9.96 retaining 73% of native enzymatic activity.

L24 ANSWER 5 OF 6 MEDLINE
ACCESSION NUMBER: 90339014 MEDLINE
DOCUMENT NUMBER: 90339014 PubMed ID: 2166134
TITLE: Spectroscopic characterization of polyethyleneglycol modified **superoxide dismutase**: 1H NMR studies on its Cu2Co2 derivative.
AUTHOR: Banci L; Bertini I; Caliceti P; Monsu Scolaro L; Schiavon O; Veronese F M
CORPORATE SOURCE: Department of Chemistry, University of Florence, Italy.
SOURCE: JOURNAL OF INORGANIC BIOCHEMISTRY, (1990 Jun) 39 (2) 149-59.
Journal code: JAR; 7905788. ISSN: 0162-0134.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199009
ENTRY DATE: Entered STN: 19901012
Last Updated on STN: 19901012
Entered Medline: 19900910

AB Spectroscopic methods have been employed in order to understand the molecular basis of the decrease in enzymatic activity of the antiinflammatory enzyme copper-zinc **superoxide dismutase** (SOD) following the **covalent** binding of polyethyleneglycol (**PEG**) chains to the protein **amino**-groups. The **PEG** modification is a general method recently proposed to improve the therapeutic index of enzymes. 1H NMR spectra on the cobalt substituted **PEG**-modified SOD, Cu2Co2-**PEG**-SOD, have been recorded. The signals are quite broad with respect to the unmodified enzyme. This has been interpreted on the basis of the effect of **molecular weight** on the linewidth. The analysis has shown that the histidine hydrogens involved in metal binding at the enzyme active site are the same in both native and **PEG**-modified SOD. Similarly, circular dichroism and absorption spectra indicate that the overall conformation of the metal clusters is not perturbed upon modification. On the other hand, azide titration shows that the affinity constant of N-3 for SOD is largely reduced upon **PEG** modification (K = 154 M-1 and 75 M-1 for the

native and modified SOD, respectively). These results indicate that the decrease in enzymatic activity upon surface modification with **PEG** is not caused by a perturbation of the active site geometry, but to a decrease in the channeling of the O2- ion towards the enzyme active site.

L24 ANSWER 6 OF 6 MEDLINE
ACCESSION NUMBER: 77187848 MEDLINE
DOCUMENT NUMBER: 77187848 PubMed ID: 16907
TITLE: Effect of **covalent** attachment of **polyethylene glycol** on immunogenicity and circulating life of bovine liver **catalase**.
AUTHOR: Abuchowski A; McCoy J R; Palczuk N C; van Es T; Davis F F
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1977 Jun 10) 252 (11) 3582-6.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197707
ENTRY DATE: Entered STN: 19900314
Last Updated on STN: 19950206
Entered Medline: 19770723

AB Methoxypolyethylene glycols of 1900 daltons (**PEG**-1900) or 5000 daltons (**PEG**-5000) were covalently attached to bovine liver **catalase** using 2,4,6-trichloro-s-triazine as the coupling agent. Rabbits were immunized by the intravenous and intramuscular routes with **catalase** modified by **covalent** attachment of **PEG** -1900 to 43% of the **amino** groups (**PEG**-1900-**catalase**). The intravenous antiserum did not yield detectable antibodies against **PEG**-1900-**catalase** or native **catalase**, as determined by Ouchterlony and complement fixation methods, whereas the intramuscular antiserum contained antibodies to both **PEG**-1900-**catalase** and **catalase**. **PEG** -1900 did not react with either antiserum. **Catalase** was prepared in which **PEG**-5000 was attached to 40% of the **amino** groups (**PEG**-5000-**catalase**). This **catalase** preparation did not react with either antiserum. **PEG**-1900-**catalase** retained 93% of its enzymatic activity; **PEG** -5000-**catalase** retained 95%. **PEG**-5000-**catalase** resisted digestion by trypsin, chymotrypsin, and a protease from Streptomyces griseus. **PEG**-1900-**catalase** and **PEG**-5000-**catalase** exhibited enhanced circulating lives in the blood of acatalasemic mice during repetitive intravenous injections. No evidence was seen of an immune response to injections of the modified enzymes. Mice injected repetitively with **PEG**-5000-**catalase** remained immune competent for unmodieied **catalase** , and no evidence of tissue or organ damage was seen.

=> d his

(FILE 'HOME' ENTERED AT 10:56:24 ON 08 NOV 2001)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:57:54 ON 08 NOV 2001

L1 60112 S PEG
L2 0 S POLYETHYLENE ADJ GLYCOL
L3 110582 S POLYETHYLENE (A) GLYCOL
L4 146676 S L1 OR L3
L5 885718 S MOLECULAR(W) WEIGHT
L6 40836 S "8000" OR "10000" OR "18000"
L7 5333 S L5 AND L6
L8 576 S L4 AND L7
L9 124212 S COVALENT OR IMMOBLI?

```
L10      247404 S (SUPEROXIDE (A)DISMUTASE?) OR CATALASE? OR (GLUTATHIONE(A)
PE
L11        1277 S L9 AND L10
L12           0 S L8 AND L11
L13         113 S L11 AND L4
L14          30 S L5 AND L13
L15          16 DUP REM L14 (14 DUPLICATES REMOVED)
L16     2362944 S WOUND OR BANDAGE OR COMPRESS? OR PLASTER? OR SHEET? OR FILM?
L17           0 S L15 AND L16
L18       70993 S ISOCYANATE?
L19           0 S L18 AND L15
L20       48865 S DIISOCYANATE?
L21           0 S L15 AND L20
L22           0 S L15 AND (UREA OR URETHANE?)
L23           6 S L15 AND AMINO
L24           6 DUP REM L23 (0 DUPLICATES REMOVED)

=> e ettner n/au

E1            1     ETTNER H U F/AU
E2            1     ETTNER I/AU
E3           26 --> ETTNER N/AU
E4           20     ETTNER NORBERT/AU
E5            3     ETTNER S/AU
E6           89     ETTNER S L/AU
E7            3     ETTNER SUSAN L/AU
E8            1     ETTNER SUSAN LOUISE/AU
E9            5     ETTNER U/AU
E10           1     ETTNGER B/AU
E11           1     ETTNIQUI A/AU
E12           4     ETTORE A/AU

=> s e3

L25          26 "ETTNER N"/AU

=> s l25 and l3

L26           0 L25 AND L3

=> s l24 and l4

L27           6 L24 AND L4

=> dup rem l27

PROCESSING COMPLETED FOR L27
L28           6 DUP REM L27 (0 DUPLICATES REMOVED)

=> d 1-6 ibib ab


L28  ANSWER 1 OF 6  SCISEARCH  COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER:    1998:12669  SCISEARCH
THE GENUINE ARTICLE: BK03M
TITLE:               Conjugation of high-molecular weight
                     poly(ethylene glycol) to cytokines:
Granulocyte-macrophage
                     colony-stimulating factors as model substrates
AUTHOR:              Sherman M R (Reprint); Williams L D; Saifer M G P; French
                     J A; Kwak L W; Oppenheim J J
CORPORATE SOURCE:    MT VIEW PHARMACEUT INC, 871-L IND PK, MENLO PK, CA 94025
                     (Reprint); NCI, FREDERICK CANC RES & DEV CTR, FREDERICK,
                     MD 21702
COUNTRY OF AUTHOR:   USA
SOURCE:              ACS SYMPOSIUM SERIES, (FEB 1997) Vol. 680, pp. 155-169.
```

Publisher: AMER CHEMICAL SOC, 1155 SIXTEENTH ST NW, WASHINGTON, DC 20036.
ISSN: 0097-6156.
DOCUMENT TYPE: General Review; Journal
LANGUAGE: English
REFERENCE COUNT: 71

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The ability of the small receptor-binding protein, recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF), to increase the abundance of certain blood cell types in mice was enhanced markedly by **covalent** attachment of a single long strand of **PEG** (30-40 kDa). Potency was not increased further by coupling a second strand. Such conjugates can be synthesized efficiently by reaction of protein **amino** groups with **PEG** propionaldehydes in the presence of NaBH3CN or with **PEG** p-nitrophenyl carbonates. Both methods have been used to prepare recombinant human GM-CSF conjugates of predetermined composition, e.g. **PEG**(1)GM-CSF and **PEG**(2)GM-CSF, in high yield. These compounds, or analogous derivatives of other cytokines, purified by ion-exchange and size-exclusion chromatography, may be suitable candidates for pharmaceutical development.

L28 ANSWER 2 OF 6 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 97:222875 SCISEARCH
THE GENUINE ARTICLE: WN017
TITLE: A simple and efficient method for preparation of monomethoxypolyethylene glycol activated with p-nitrophenylchloroformate and its application to modification of L-asparaginase
AUTHOR: Kito M; Miron T; Wilchek M; Kojima N; Ohishi N; Yagi K (Reprint)
CORPORATE SOURCE: INST APPL BIOCHEM, YAGI MEM PK, GIFU 50501, JAPAN (Reprint); INST APPL BIOCHEM, GIFU 50501, JAPAN; WEIZMANN INST SCI, DEPT BIOPHYS, IL-76100 REHOVOT, ISRAEL
COUNTRY OF AUTHOR: JAPAN; ISRAEL
SOURCE: JOURNAL OF CLINICAL BIOCHEMISTRY AND NUTRITION, (SEP 1996)
Vol. 21, No. 2, pp. 101-111.
Publisher: INST APPLIED BIOCHEMISTRY, YAGI MEMORIAL PARK, MITAKE GIFU 505-01, JAPAN.
ISSN: 0912-0009.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 28

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB An improved, simple and efficient method for preparation of monomethoxypolyethylene glycol (**PEG**) activated with p-nitrophenylchloroformate (PNP-**PEG**) and its use as a potent modifier of protein under mild conditions are described. Modification of bovine serum albumin with PNP-**PEG** was compared with that done with **PEG** activated with N,N'-carbonyldiimidazole or cyanuric chloride. The reaction of **PEG**, activated with either p-nitrophenylchloroformate or cyanuric chloride, with bovine serum albumin at 4 degrees C reached a plateau within 1 h, whereas protein modification using **PEG** activated,with N,N'-carbonyldiimidazole was rather slow and gave a low yield. The remaining activity of L-asparaginase modified with PNP-**PEG** was much higher than that of the enzyme modified to the same degree with **PEG** activated with cyanuric chloride. At a 20 molar excess of PNP-**PEG** having a **molecular weight** of 5,000, 55% of the free **amino** acid groups were modified at 4 degrees C for 2 h, and the modified enzyme still had 33% residual enzyme activity. Immunochemical studies showed that

the highly modified enzyme (67% modification with 18% residual enzyme activity) had lost its immunogenicity and had become much less sensitive to protease digestion.

L28 ANSWER 3 OF 6 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1995:16065 BIOSIS
DOCUMENT NUMBER: PREV199598030365
TITLE: Acylation of **amino** functions of proteins with monomethoxypoly (ethylene glycol)-N-succinimide carbonate.
AUTHOR(S): Nijs, Michelle; Gelbcke, Michel; Azarkan, Mohamed; Brygier, Jeanne; Guermant, Claude; Baeyens-Volant, Danielle; Musu, Tony; Paul, Claudine; Looze, Yvan (1)
CORPORATE SOURCE: (1) Protein Chem. Unit, Fac. Med., Univ. Brussels, Brussels Belgium
SOURCE: Applied Biochemistry and Biotechnology, (1994) Vol. 49, No. 1, pp. 75-91.
ISSN: 0273-2289.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Monomethoxypoly(ethylene glycol)-N-succinimide carbonate (SC-**PEG**) was used to prepare **PEG**-lysozyme, **PEG**-papaya proteinase 111, **PEG-catalase**, and **PEG**-lactoperoxidase conjugates. SC-**PEG** produced extensively modified enzymes under mild conditions (pH 7.0; 25 degree C) within a couple of hours. **PEG**-enzyme conjugates showed equal or even greater specific activity provided that low-**molecular-weight** substrates were used to evaluate the biological activities. However, papaya proteinase III and lysozyme lost their proteolytic and bacteriolytic activities, respectively, on conjugation with **PEG**. This was most probably because of steric factors, since no drastic conformational changes could be detected after conjugation of these enzymes with **PEG** chains. Unlike these enzymes, the secondary structures of the two hemoproteins were somewhat affected by the **covalent** attachment of **PEG** chains as shown by FTIR experiments. These results confirmed the potential usefulness of SC-**PEG**, for which a novel route of synthesis making use of N,N'-disuccinimidyl carbonate was described.

L28 ANSWER 4 OF 6 MEDLINE
ACCESSION NUMBER: 93289947 MEDLINE
DOCUMENT NUMBER: 93289947 PubMed ID: 8512060
TITLE: Reagents for the preparation of chromophorically labeled **polyethylene glycol**-protein conjugates.
AUTHOR: Ladd D L; Snow R A
CORPORATE SOURCE: Medicinal Chemistry Department, Sterling Winthrop Pharmaceuticals Research Division, Sterling Winthrop Inc., Malvern, Pennsylvania 19355.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1993 May 1) 210 (2) 258-61.
Journal code: 4NK; 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19930723
Last Updated on STN: 19930723
Entered Medline: 19930709
AB We have developed a new class of reagents (2) for the **covalent** attachment of **polyethylene glycol** to proteins. These reagents (2) are the monomethoxypolyethylene glycol esters of 4-fluoro-3-nitrobenzoic acid. The reaction of 2 with lysine epsilon-**amino** groups produces a chromophore which can be used to

quantitate the **polyethylene glycol** to protein molar ratio. Bovine (Zn,Cu) **superoxide dismutase** was used as a model protein for conjugation with 2. When monomethoxypolyethylene glycol of average **molecular weight** 2105 was used, a conjugate was obtained with a **polyethylene glycol** to protein molar ratio of 8.88 retaining 100% of native enzymatic activity; monomethoxypolyethylene glycol of average **molecular weight** 5210 yielded a conjugate with a **polyethylene glycol** to protein molar ratio of 9.96 retaining 73% of native enzymatic activity.

L28 ANSWER 5 OF 6 MEDLINE
ACCESSION NUMBER: 90339014 MEDLINE
DOCUMENT NUMBER: 90339014 PubMed ID: 2166134
TITLE: Spectroscopic characterization of polyethyleneglycol modified **superoxide dismutase**: 1H NMR studies on its Cu2Co2 derivative.
AUTHOR: Banci L; Bertini I; Caliceti P; Monsu Scolaro L; Schiavon O; Veronese F M
CORPORATE SOURCE: Department of Chemistry, University of Florence, Italy.
SOURCE: JOURNAL OF INORGANIC BIOCHEMISTRY, (1990 Jun) 39 (2) 149-59.
Journal code: JAR; 7905788. ISSN: 0162-0134.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199009
ENTRY DATE: Entered STN: 19901012
Last Updated on STN: 19901012
Entered Medline: 19900910

AB Spectroscopic methods have been employed in order to understand the molecular basis of the decrease in enzymatic activity of the antiinflammatory enzyme copper-zinc **superoxide dismutase** (SOD) following the **covalent** binding of polyethyleneglycol (**PEG**) chains to the protein **amino**-groups. The **PEG** modification is a general method recently proposed to improve the therapeutic index of enzymes. 1H NMR spectra on the cobalt substituted **PEG**-modified SOD, Cu2Co2-**PEG**-SOD, have been recorded. The signals are quite broad with respect to the unmodified enzyme. This has been interpreted on the basis of the effect of **molecular weight** on the linewidth. The analysis has shown that the histidine hydrogens involved in metal binding at the enzyme active site are the same in both native and **PEG**-modified SOD. Similarly, circular dichroism and absorption spectra indicate that the overall conformation of the metal clusters is not perturbed upon modification. On the other hand, azide titration shows that the affinity constant of N-3 for SOD is largely reduced upon **PEG** modification (K = 154 M-1 and 75 M-1 for the native and modified SOD, respectively). These results indicate that the decrease in enzymatic activity upon surface modification with **PEG** is not caused by a perturbation of the active site geometry, but to a decrease in the channeling of the O2- ion towards the enzyme active site.

L28 ANSWER 6 OF 6 MEDLINE
ACCESSION NUMBER: 77187848 MEDLINE
DOCUMENT NUMBER: 77187848 PubMed ID: 16907
TITLE: Effect of **covalent** attachment of **polyethylene glycol** on immunogenicity and circulating life of bovine liver **catalase**.
AUTHOR: Abuchowski A; McCoy J R; Palczuk N C; van Es T; Davis F F
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1977 Jun 10) 252 (11) 3582-6.
Journal code: HIV; 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197707
ENTRY DATE: Entered STN: 19900314
Last Updated on STN: 19950206
Entered Medline: 19770723

AB Methoxypolyethylene glycols of 1900 daltons (**PEG**-1900) or 5000 daltons (**PEG**-5000) were covalently attached to bovine liver **catalase** using 2,4,6-trichloro-s-triazine as the coupling agent. Rabbits were immunized by the intravenous and intramuscular routes with **catalase** modified by **covalent** attachment of **PEG** -1900 to 43% of the **amino** groups (**PEG**-1900-**catalase**). The intravenous antiserum did not yield detectable antibodies against **PEG**-1900-**catalase** or native **catalase**, as determined by Ouchterlony and complement fixation methods, whereas the intramuscular antiserum contained antibodies to both **PEG**-1900-**catalase** and **catalase**. **PEG** -1900 did not react with either antiserum. **Catalase** was prepared in which **PEG**-5000 was attached to 40% of the **amino** groups (**PEG**-5000-**catalase**). This **catalase** preparation did not react with either antiserum. **PEG**-1900-**catalase** retained 93% of its enzymatic activity; **PEG** -5000-**catalase** retained 95%. **PEG**-5000-**catalase** resisted digestion by trypsin, chymotrypsin, and a protease from Streptomyces griseus. **PEG**-1900-**catalase** and **PEG**-5000-**catalase** exhibited enhanced circulating lives in the blood of acatalasemic mice during repetitive intravenous injections. No evidence was seen of an immune response to injections of the modified enzymes. Mice injected repetitively with **PEG**-5000-**catalase** remained immune competent for unmodieied **catalase** , and no evidence of tissue or organ damage was seen.

=> d his

(FILE 'HOME' ENTERED AT 10:56:24 ON 08 NOV 2001)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 10:57:54 ON 08 NOV 2001

```
L1        60112 S PEG
L2            0 S POLYETHYLENE ADJ GLYCOL
L3       110582 S POLYETHYLENE (A) GLYCOL
L4       146676 S L1 OR L3
L5       885718 S MOLECULAR(W) WEIGHT
L6        40836 S "8000" OR "10000" OR "18000"
L7         5333 S L5 AND L6
L8          576 S L4 AND L7
L9       124212 S COVALENT OR IMMOBLI?
L10      247404 S (SUPEROXIDE (A)DISMUTASE?) OR CATALASE? OR (GLUTATHIONE(A)
PE
L11        1277 S L9 AND L10
L12           0 S L8 AND L11
L13         113 S L11 AND L4
L14          30 S L5 AND L13
L15          16 DUP REM L14 (14 DUPLICATES REMOVED)
L16     2362944 S WOUND OR BANDAGE OR COMPRESS? OR PLASTER? OR SHEET? OR FILM?
L17           0 S L15 AND L16
L18       70993 S ISOCYANATE?
L19           0 S L18 AND L15
L20       48865 S DIISOCYANATE?
L21           0 S L15 AND L20
L22           0 S L15 AND (UREA OR URETHANE?)
L23           6 S L15 AND AMINO
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L24            6 DUP REM L23 (0 DUPLICATES REMOVED)
                 E ETT[redacted] N/AU
L25           26 S E3
L26            0 S L25 AND L3
L27            6 S L24 AND L4
L28            6 DUP REM L27 (0 DUPLICATES REMOVED)

=>  s l28 and l6

L29            0 L28 AND L6

=> e schink m/au

E1             3      SCHINK KARL/AU
E2             2      SCHINK L/AU
E3            34 --> SCHINK M/AU
E4             1      SCHINK M J/AU
E5            10      SCHINK M M/AU
E6             3      SCHINK MAGDOLNA/AU
E7             1      SCHINK MAGDOLNA HORVAY/AU
E8             6      SCHINK MICHAEL/AU
E9             2      SCHINK MYRA/AU
E10            1      SCHINK N/AU
E11           46      SCHINK NORBERT/AU
E12            3      SCHINK NORBERT F/AU

=> s e3

L30           34 "SCHINK M"/AU

=> s l4 and l30

L31            0 L4 AND L30

=> e schreiber J/au

E1             2      SCHREIBER IRIS/AU
E2             2      SCHREIBER IRMELA/AU
E3           811 --> SCHREIBER J/AU
E4             4      SCHREIBER J A/AU
E5            37      SCHREIBER J B/AU
E6             8      SCHREIBER J C/AU
E7           116      SCHREIBER J D/AU
E8             8      SCHREIBER J E/AU
E9            12      SCHREIBER J F/AU
E10            3      SCHREIBER J F JR/AU
E11           60      SCHREIBER J H/AU
E12            7      SCHREIBER J J/AU

=> s e3

L32          811 "SCHREIBER J"/AU

=> s l32 and l4

L33            0 L32 AND L4

=> e meier w/au

E1             9      MEIER VOLKER/AU
E2             1      MEIER VOLKER VM/AU
E3          1206 --> MEIER W/AU
E4            45      MEIER W A/AU
E5             1      MEIER W B/AU
E6             3      MEIER W D/AU
E7            83      MEIER W E/AU
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E8            1      MEIER W G/AU
E9            1      MEIER W H/AU
E10           2      MEIER W J/AU
E11           3      MEIER W JEFF/AU
E12          24      MEIER W L/AU

=> s e3

L34       1206 "MEIER W"/AU

=> s l4 and l34

L35          3 L4 AND L34

=> dup rem l35

PROCESSING COMPLETED FOR L35
L36           2 DUP REM L35 (1 DUPLICATE REMOVED)

=> d 1-2 ibib ab


L36  ANSWER 1 OF 2  BIOSIS  COPYRIGHT 2001 BIOSIS       DUPLICATE 1
ACCESSION NUMBER:   2000:351159  BIOSIS
DOCUMENT NUMBER:    PREV200000351159
TITLE:              Treatment of progressive and recurrent ovarian cancer.
AUTHOR(S):          Meier, W. (1); Gropp, M.; Burges, A.; Hepp, H.
CORPORATE SOURCE:   (1) Frauenklinik, Evangelisches Krankenhaus,
                    Kirchfeldstrasse 40, D-40217, Duesseldorf Germany
SOURCE:             Onkologie, (April, 2000) Vol. 23, No. Suppl. 2, pp. 35-39.
                    print.
                    ISSN: 0378-584X.
DOCUMENT TYPE:      Article
LANGUAGE:           German
SUMMARY LANGUAGE:   English; German
AB   Secondary surgery after failure of primary treatment is a promising and
     reasonable option only for patients with a relapse-free interval of at
     least 6-12 months who should have ideally achieved a tumor-free status
     after primary therapy. As after primary surgery, size of residual tumor
is
     the most significant predictor of survival after secondary surgery. Even
     in the case of multiple tumor sites, complete removal of the tumor can be
     achieved in nearly 30% of the patients. Treatment results are much better
     in specialized oncology centers with optimal interdisciplinary
cooperation
     compared with smaller institutions. Chemotherapy can be used both for
     consolidation after successful secondary surgery and for palliation in
     patients with inoperable recurrent disease. Since paclitaxel has been
     integrated into first-line chemotherapy, there is no defined standard for
     second-line chemotherapy. Several cytotoxic agents have shown moderate
     activity in this setting, including treosulfan, epirubicin, and newer
     agents such as topotecan, gemcitabine, vinorelbine, and PEG(
     polyethylene glycol)-liposomal doxorubicin. Thus, the
     German Arbeitsgemeinschaft Gynaekologische Onkologie (AGO) has initiated
     several randomized studies in patients with recurrent ovarian cancer in
     order to define new standards for second-line chemotherapy.

L36  ANSWER 2 OF 2  HCAPLUS  COPYRIGHT 2001 ACS
ACCESSION NUMBER:        1997:502004  HCAPLUS
DOCUMENT NUMBER:         127:177044
TITLE:                   Structure of W/O-microemulsion-templated polymer
                         networks
AUTHOR(S):               Meier, W.
CORPORATE SOURCE:        Institut Physikalische Chemie, Basel, CH-4056, Switz.
SOURCE:                  Colloid Polym. Sci. (1997), 275(6), 530-536
                         CODEN: CPMSB6; ISSN: 0303-402X
```

PUBLISHER: Steinkopff
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The droplet phase of a water-in-oil microemulsion is used as a matrix to create amphiphilic polymer network structures from a poly(oxyethylene)-poly(isoprene)-poly(oxyethylene) triblock copolymer bearing polymerizable methacrylate end groups. Cond. measurements and freeze fracture electron microscopy are used to obtain information about the structure of these gels. The droplet structure of the underlying microemulsion is only preserved for low droplet and polymer concn. For higher concns. the crosslinking reaction can induce the formation of a bicontinuous structure.

=> d 1-2 kwic

L36 ANSWER 1 OF 2 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 1
AU **Meier, W. (1)**; Gropp, M.; Burges, A.; Hepp, H.
AB. . . agents have shown moderate activity in this setting, including treosulfan, epirubicin, and newer agents such as topotecan, gemcitabine, vinorelbine, and **PEG(polyethylene glycol**
)-liposomal doxorubicin. Thus, the German Arbeitsgemeinschaft Gynaekologische Onkologie (AGO) has initiated several randomized studies in patients with recurrent ovarian cancer in. . .
IT . . .
disease, recurrence, reproductive system disease/female, treatment; secondary surgery: surgical method, therapeutic method
IT Chemicals & Biochemicals
doxorubicin: antineoplastic - drug, **polyethylene glycol**-liposomal formulation; epirubicin: antineoplastic - drug; gemcitabine: antineoplastic - drug; paclitaxel: antineoplastic - drug; topotecan: antineoplastic - drug; treosulfan: antineoplastic -. . .

L36 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2001 ACS
AU **Meier, W.**
ST morphol **polyethylene glycol** polyisoprene microemulsion templated

=> e sauer M/au

E1	1		SAUER LOUIS W/AU
E2	1		SAUER LUDWIG/AU
E3	550	-->	SAUER M/AU
E4	34		SAUER M A/AU
E5	1		SAUER M B/AU
E6	81		SAUER M C/AU
E7	28		SAUER M C JR/AU
E8	6		SAUER M C V/AU
E9	6		SAUER M E/AU
E10	1		SAUER M H M/AU
E11	176		SAUER M J/AU
E12	25		SAUER M K/AU

=> s e3

L37 550 "SAUER M"/AU

=> s l4 and l37

L38 0 L4 AND L37

=> d his

```
     (FILE 'HOME' ENTERED AT 10:56:24 ON 08 NOV 2001)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 10:57:54 ON 08 NOV 2001
L1         60112 S PEG
L2             0 S POLYETHYLENE ADJ GLYCOL
L3        110582 S POLYETHYLENE (A) GLYCOL
L4        146676 S L1 OR L3
L5        885718 S MOLECULAR(W) WEIGHT
L6         40836 S "8000" OR "10000" OR "18000"
L7          5333 S L5 AND L6
L8           576 S L4 AND L7
L9        124212 S COVALENT OR IMMOBLI?
L10       247404 S (SUPEROXIDE (A)DISMUTASE?) OR CATALASE? OR (GLUTATHIONE(A)
PE
L11         1277 S L9 AND L10
L12            0 S L8 AND L11
L13          113 S L11 AND L4
L14           30 S L5 AND L13
L15           16 DUP REM L14 (14 DUPLICATES REMOVED)
L16      2362944 S WOUND OR BANDAGE OR COMPRESS? OR PLASTER? OR SHEET? OR FILM?
L17            0 S L15 AND L16
L18        70993 S ISOCYANATE?
L19            0 S L18 AND L15
L20        48865 S DIISOCYANATE?
L21            0 S L15 AND L20
L22            0 S L15 AND (UREA OR URETHANE?)
L23            6 S L15 AND AMINO
L24            6 DUP REM L23 (0 DUPLICATES REMOVED)
                 E ETTNER N/AU
L25           26 S E3
L26            0 S L25 AND L3
L27            6 S L24 AND L4
L28            6 DUP REM L27 (0 DUPLICATES REMOVED)
L29            0 S L28 AND L6
                 E SCHINK M/AU
L30           34 S E3
L31            0 S L4 AND L30
                 E SCHREIBER J/AU
L32          811 S E3
L33            0 S L32 AND L4
                 E MEIER W/AU
L34         1206 S E3
L35            3 S L4 AND L34
L36            2 DUP REM L35 (1 DUPLICATE REMOVED)
                 E SAUER M/AU
L37          550 S E3
L38            0 S L4 AND L37

=> log hold

COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                   126.41     126.86

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                       ENTRY    SESSION
CA SUBSCRIBER PRICE                                    -1.76      -1.76

 SESSION WILL BE HELD FOR 60 MINUTES
STN INTERNATIONAL SESSION SUSPENDED AT 11:38:08 ON 08 NOV 2001
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L21             0 S L15 AND L20
L22             0 S L15 AND (UREA OR URETHANE?)
L23             6 S L15 AND AMINO
L24             6 DUP REM L23 (0 DUPLICATES REMOVED)

=> e ettner n/au

E1              1     ETTNER H U F/AU
E2              1     ETTNER I/AU
E3             26 --> ETTNER N/AU
E4             20     ETTNER NORBERT/AU
E5              3     ETTNER S/AU
E6             89     ETTNER S L/AU
E7              3     ETTNER SUSAN L/AU
E8              1     ETTNER SUSAN LOUISE/AU
E9              5     ETTNER U/AU
E10             1     ETTNGER B/AU
E11             1     ETTNIQUI A/AU
E12             4     ETTORE A/AU

=> s e3

L25            26 "ETTNER N"/AU

=> s l25 and l3

L26             0 L25 AND L3

=> s l24 and l4

L27             6 L24 AND L4

=> dup rem l27

PROCESSING COMPLETED FOR L27
L28             6 DUP REM L27 (0 DUPLICATES REMOVED)

=> d 1-6 ibib ab


L28  ANSWER 1 OF 6  SCISEARCH  COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER:    1998:12669  SCISEARCH
THE GENUINE ARTICLE: BK03M
TITLE:               Conjugation of high-molecular weight
                     poly(ethylene glycol) to cytokines:
Granulocyte-macrophage
                     colony-stimulating factors as model substrates
AUTHOR:              Sherman M R (Reprint); Williams L D; Saifer M G P; French
                     J A; Kwak L W; Oppenheim J J
CORPORATE SOURCE:    MT VIEW PHARMACEUT INC, 871-L IND PK, MENLO PK, CA 94025
                     (Reprint); NCI, FREDERICK CANC RES & DEV CTR, FREDERICK,
                     MD 21702
COUNTRY OF AUTHOR:   USA
SOURCE:              ACS SYMPOSIUM SERIES, (FEB 1997) Vol. 680, pp. 155-169.
                     Publisher: AMER CHEMICAL SOC, 1155 SIXTEENTH ST NW,
                     WASHINGTON, DC 20036.
                     ISSN: 0097-6156.
DOCUMENT TYPE:       General Review; Journal
LANGUAGE:            English
REFERENCE COUNT:     71
```

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The ability of the small receptor-binding protein, recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF), to increase the abundance of certain blood cell types in mice was enhanced markedly by **covalent** attachment of a single long strand of **PEG** (30-40 kDa). Potency was not increased further by coupling a second strand. Such conjugates can be synthesized efficiently by reaction of protein **amino** groups with **PEG** propionaldehydes in the presence of NaBH3CN or with **PEG** p-nitrophenyl carbonates. Both methods have been used to prepare recombinant human GM-CSF conjugates of predetermined composition, e.g. **PEG**(1)GM-CSF and **PEG**(2)GM-CSF, in high yield. These compounds, or analogous derivatives of other cytokines, purified by ion-exchange and size-exclusion chromatography, may be suitable candidates for pharmaceutical development.

L28 ANSWER 2 OF 6 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 97:222875 SCISEARCH
THE GENUINE ARTICLE: WN017
TITLE: A simple and efficient method for preparation of monomethoxypolyethylene glycol activated with p-nitrophenylchloroformate and its application to modification of L-asparaginase
AUTHOR: Kito M; Miron T; Wilchek M; Kojima N; Ohishi N; Yagi K (Reprint)
CORPORATE SOURCE: INST APPL BIOCHEM, YAGI MEM PK, GIFU 50501, JAPAN (Reprint); INST APPL BIOCHEM, GIFU 50501, JAPAN; WEIZMANN INST SCI, DEPT BIOPHYS, IL-76100 REHOVOT, ISRAEL
COUNTRY OF AUTHOR: JAPAN; ISRAEL
SOURCE: JOURNAL OF CLINICAL BIOCHEMISTRY AND NUTRITION, (SEP 1996)
Vol. 21, No. 2, pp. 101-111.
Publisher: INST APPLIED BIOCHEMISTRY, YAGI MEMORIAL PARK, MITAKE GIFU 505-01, JAPAN.
ISSN: 0912-0009.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 28

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB An improved, simple and efficient method for preparation of monomethoxypolyethylene glycol (**PEG**) activated with p-nitrophenylchloroformate (PNP-**PEG**) and its use as a potent modifier of protein under mild conditions are described. Modification of bovine serum albumin with PNP-**PEG** was compared with that done with **PEG** activated with N,N'-carbonyldiimidazole or cyanuric chloride. The reaction of **PEG**, activated with either p-nitrophenylchloroformate or cyanuric chloride, with bovine serum albumin at 4 degrees C reached a plateau within 1 h, whereas protein modification using **PEG** activated,with N,N'-carbonyldiimidazole was rather slow and gave a low yield. The remaining activity of L-asparaginase modified with PNP-**PEG** was much higher than that of the enzyme modified to the same degree with **PEG** activated with cyanuric chloride. At a 20 molar excess of PNP-**PEG** having a **molecular weight** of 5,000, 55% of the free **amino** acid groups were modified at 4 degrees C for 2 h, and the modified enzyme still had 33% residual enzyme activity. Immunochemical studies showed that the highly modified enzyme (67% modification with 18% residual enzyme activity) had lost its immunogenicity and had become much less sensitive to protease digestion.

L28 ANSWER 3 OF 6 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1995:16065 BIOSIS

DOCUMENT NUMBER: PREV199598030365
TITLE: Activation of **amino** functions of proteins with monomethoxypoly (ethylene glycol)-N-succinimide carbonate.
AUTHOR(S): Nijs, Michelle; Gelbcke, Michel; Azarkan, Mohamed; Brygier, Jeanne; Guermant, Claude; Baeyens-Volant, Danielle; Musu, Tony; Paul, Claudine; Looze, Yvan (1)
CORPORATE SOURCE: (1) Protein Chem. Unit, Fac. Med., Univ. Brussels, Brussels Belgium
SOURCE: Applied Biochemistry and Biotechnology, (1994) Vol. 49, No. 1, pp. 75-91.
ISSN: 0273-2289.
DOCUMENT TYPE: Article
LANGUAGE: English

AB Monomethoxypoly(ethylene glycol)-N-succinimide carbonate (SC-**PEG**) was used to prepare **PEG**-lysozyme, **PEG**-papaya proteinase 111, **PEG-catalase**, and **PEG**-lactoperoxidase conjugates. SC-**PEG** produced extensively modified enzymes under mild conditions (pH 7.0; 25 degree C) within a couple of hours. **PEG**-enzyme conjugates showed equal or even greater specific activity provided that low-**molecular-weight** substrates were used to evaluate the biological activities. However, papaya proteinase III and lysozyme lost their proteolytic and bacteriolytic activities, respectively, on conjugation with **PEG**. This was most probably because of steric factors, since no drastic conformational changes could be detected after conjugation of these enzymes with **PEG** chains. Unlike these enzymes, the secondary structures of the two hemoproteins were somewhat affected by the **covalent** attachment of **PEG** chains as shown by FTIR experiments. These results confirmed the potential usefulness of SC-**PEG**, for which a novel route of synthesis making use of N,N'-disuccinimidyl carbonate was described.

L28 ANSWER 4 OF 6 MEDLINE
ACCESSION NUMBER: 93289947 MEDLINE
DOCUMENT NUMBER: 93289947 PubMed ID: 8512060
TITLE: Reagents for the preparation of chromophorically labeled **polyethylene glycol**-protein conjugates.
AUTHOR: Ladd D L; Snow R A
CORPORATE SOURCE: Medicinal Chemistry Department, Sterling Winthrop Pharmaceuticals Research Division, Sterling Winthrop Inc., Malvern, Pennsylvania 19355.
SOURCE: ANALYTICAL BIOCHEMISTRY, (1993 May 1) 210 (2) 258-61.
Journal code: 4NK; 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199307
ENTRY DATE: Entered STN: 19930723
Last Updated on STN: 19930723
Entered Medline: 19930709

AB We have developed a new class of reagents (2) for the **covalent** attachment of **polyethylene glycol** to proteins. These reagents (2) are the monomethoxypolyethylene glycol esters of 4-fluoro-3-nitrobenzoic acid. The reaction of 2 with lysine epsilon-**amino** groups produces a chromophore which can be used to quantitate the **polyethylene glycol** to protein molar ratio. Bovine (Zn, Cu) **superoxide dismutase** was used as a model protein for conjugation with 2. When monomethoxypolyethylene glycol of average **molecular weight** 2105 was used, a conjugate was obtained with a **polyethylene glycol** to protein molar ratio of 8.88 retaining 100% of native enzymatic activity;

monomethoxypolyethylene glycol of average **molecular weight** 5210 yiel[redacted] a conjugate with a **polyethyle[redacted] glycol** to protein molar ratio of 9.96 retaining 73% of native enzymatic activity.

L28 ANSWER 5 OF 6 MEDLINE
ACCESSION NUMBER: 90339014 MEDLINE
DOCUMENT NUMBER: 90339014 PubMed ID: 2166134
TITLE: Spectroscopic characterization of polyethyleneglycol modified **superoxide dismutase**: 1H NMR studies on its Cu2Co2 derivative.
AUTHOR: Banci L; Bertini I; Caliceti P; Monsu Scolaro L; Schiavon O; Veronese F M
CORPORATE SOURCE: Department of Chemistry, University of Florence, Italy.
SOURCE: JOURNAL OF INORGANIC BIOCHEMISTRY, (1990 Jun) 39 (2) 149-59.
Journal code: JAR; 7905788. ISSN: 0162-0134.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199009
ENTRY DATE: Entered STN: 19901012
Last Updated on STN: 19901012
Entered Medline: 19900910

AB Spectroscopic methods have been employed in order to understand the molecular basis of the decrease in enzymatic activity of the antiinflammatory enzyme copper-zinc **superoxide dismutase** (SOD) following the **covalent** binding of polyethyleneglycol (**PEG**) chains to the protein **amino**-groups. The **PEG** modification is a general method recently proposed to improve the therapeutic index of enzymes. 1H NMR spectra on the cobalt substituted **PEG**-modified SOD, Cu2Co2-**PEG**-SOD, have been recorded. The signals are quite broad with respect to the unmodified enzyme. This has been interpreted on the basis of the effect of **molecular weight** on the linewidth. The analysis has shown that the histidine hydrogens involved in metal binding at the enzyme active site are the same in both native and **PEG**-modified SOD. Similarly, circular dichroism and absorption spectra indicate that the overall conformation of the metal clusters is not perturbed upon modification. On the other hand, azide titration shows that the affinity constant of N-3 for SOD is largely reduced upon **PEG** modification (K = 154 M-1 and 75 M-1 for the native and modified SOD, respectively). These results indicate that the decrease in enzymatic activity upon surface modification with **PEG** is not caused by a perturbation of the active site geometry, but to a decrease in the channeling of the O2- ion towards the enzyme active site.

L28 ANSWER 6 OF 6 MEDLINE
ACCESSION NUMBER: 77187848 MEDLINE
DOCUMENT NUMBER: 77187848 PubMed ID: 16907
TITLE: Effect of **covalent** attachment of **polyethylene glycol** on immunogenicity and circulating life of bovine liver **catalase**.
AUTHOR: Abuchowski A; McCoy J R; Palczuk N C; van Es T; Davis F F
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1977 Jun 10) 252 (11) 3582-6.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197707
ENTRY DATE: Entered STN: 19900314

Last Updated on STN: 19950206
Entered Medline: 19770723

AB Methoxypolyethylene glycols of 1900 daltons (**PEG**-1900) or 5000 daltons (**PEG**-5000) were covalently attached to bovine liver **catalase** using 2,4,6-trichloro-s-triazine as the coupling agent. Rabbits were immunized by the intravenous and intramuscular routes with **catalase** modified by **covalent** attachment of **PEG** -1900 to 43% of the **amino** groups (**PEG**-1900-**catalase**). The intravenous antiserum did not yield detectable antibodies against **PEG**-1900-**catalase** or native **catalase**, as determined by Ouchterlony and complement fixation methods, whereas the intramuscular antiserum contained antibodies to both **PEG**-1900-**catalase** and **catalase**. **PEG** -1900 did not react with either antiserum. **Catalase** was prepared in which **PEG**-5000 was attached to 40% of the **amino** groups (**PEG**-5000-**catalase**). This **catalase** preparation did not react with either antiserum. **PEG**-1900-**catalase** retained 93% of its enzymatic activity; **PEG** -5000-**catalase** retained 95%. **PEG**-5000-**catalase** resisted digestion by trypsin, chymotrypsin, and a protease from Streptomyces griseus. **PEG**-1900-**catalase** and **PEG**-5000-**catalase** exhibited enhanced circulating lives in the blood of acatalasemic mice during repetitive intravenous injections. No evidence was seen of an immune response to injections of the modified enzymes. Mice injected repetitively with **PEG**-5000-**catalase** remained immune competent for unmodieied **catalase** , and no evidence of tissue or organ damage was seen.

L28 ANSWER 1 OF 6 SCISEARCH COPYRIGHT 2001 ISI (R)
ACCESSION NUMBER: 1998:12669 SCISEARCH
THE GENUINE ARTICLE: BK03M
TITLE: Conjugation of high-**molecular weight** poly(ethylene glycol) to cytokines: Granulocyte-macrophage colony-stimulating factors as model substrates
AUTHOR: Sherman M R (Reprint); Williams L D; Saifer M G P; French J A; Kwak L W; Oppenheim J J
CORPORATE SOURCE: MT VIEW PHARMACEUT INC, 871-L IND PK, MENLO PK, CA 94025 (Reprint); NCI, FREDERICK CANC RES & DEV CTR, FREDERICK, MD 21702
COUNTRY OF AUTHOR: USA
SOURCE: ACS SYMPOSIUM SERIES, (FEB 1997) Vol. 680, pp. 155-169. Publisher: AMER CHEMICAL SOC, 1155 SIXTEENTH ST NW, WASHINGTON, DC 20036.
ISSN: 0097-6156.
DOCUMENT TYPE: General Review; Journal
LANGUAGE: English
REFERENCE COUNT: 71

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The ability of the small receptor-binding protein, recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF), to increase the abundance of certain blood cell types in mice was enhanced markedly by **covalent** attachment of a single long strand of **PEG** (30-40 kDa). Potency was not increased further by coupling a second strand. Such conjugates can be synthesized efficiently by reaction of protein **amino** groups with **PEG** propionaldehydes in the presence of NaBH3CN or with **PEG** p-nitrophenyl carbonates. Both methods have been used to prepare recombinant human GM-CSF conjugates of predetermined composition, e.g. **PEG**(1)GM-CSF and **PEG**(2)GM-CSF, in high yield. These compounds, or analogous derivatives of other cytokines, purified by ion-exchange and size-exclusion chromatography, may be suitable candidates for pharmaceutical development.

09/493,887

L24 ANSWER 5 OF 6 MEDLINE
ACCESSION NUMBER: 90339014 MEDLINE
DOCUMENT NUMBER: 90339014 PubMed ID: 2166134
TITLE: Spectroscopic characterization of polyethyleneglycol modified **superoxide dismutase**: 1H NMR studies on its Cu2Co2 derivative.
AUTHOR: Banci L; Bertini I; Caliceti P; Monsu Scolaro L; Schiavon O; Veronese F M
CORPORATE SOURCE: Department of Chemistry, University of Florence, Italy.
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Journal code: JAR; 7905788. ISSN: 0162-0134.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199009
ENTRY DATE: Entered STN: 19901012
Last Updated on STN: 19901012
Entered Medline: 19900910

AB Spectroscopic methods have been employed in order to understand the molecular basis of the decrease in enzymatic activity of the antiinflammatory enzyme copper-zinc **superoxide dismutase** (SOD) following the **covalent** binding of polyethyleneglycol (**PEG**) chains to the protein **amino**-groups. The **PEG** modification is a general method recently proposed to improve the therapeutic index of enzymes. 1H NMR spectra on the cobalt substituted **PEG**-modified SOD, Cu2Co2-**PEG**-SOD, have been recorded. The signals are quite broad with respect to the unmodified enzyme. This has been interpreted on the basis of the effect of **molecular weight** on the linewidth. The analysis has shown that the histidine hydrogens involved in metal binding at the enzyme active site are the same in both native and **PEG**-modified SOD. Similarly, circular dichroism and absorption spectra indicate that the overall conformation of the metal clusters is not perturbed upon modification. On the other hand, azide titration shows that the affinity constant of N-3 for SOD is largely reduced upon **PEG** modification (K = 154 M-1 and 75 M-1 for the native and modified SOD, respectively). These results indicate that the decrease in enzymatic activity upon surface modification with **PEG** is not caused by a perturbation of the active site geometry, but to a decrease in the channeling of the O2- ion towards the enzyme active site.

L24 ANSWER 6 OF 6 MEDLINE
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DOCUMENT NUMBER: 77187848 PubMed ID: 16907
TITLE: Effect of **covalent** attachment of **polyethylene glycol** on immunogenicity and circulating life of bovine liver **catalase**.
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SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1977 Jun 10) 252 (11) 3582-6.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 197707
ENTRY DATE: Entered STN: 19900314
Last Updated on STN: 19950206
Entered Medline: 19770723

AB Methoxypolyethylene glycols of 1900 daltons (**PEG**-1900) or 5000 daltons (**PEG**-5000) were covalently attached to bovine liver **catalase** using 2,4,6-trichloro-s-triazine as the coupling agent. Rabbits were immunized by the intravenous and intramuscular routes with **catalase** modified by **covalent** attachment of **PEG** -1900 to 43% of the **amino** groups (**PEG**-1900-**catalase**). The intravenous antiserum did not yield detectable antibodies against **PEG**-1900-**catalase** or native **catalase**, as determined by Ouchterlony and complement fixation methods, whereas the intramuscular antiserum contained antibodies to both **PEG**-1900-**catalase** and **catalase**. **PEG** -1900 did not react with either antiserum. **Catalase** was prepared in which **PEG**-5000 was attached to 40% of the **amino** groups (**PEG**-5000-**catalase**). This **catalase** preparation did not react with either antiserum. **PEG**-1900-**catalase** retained 93% of its enzymatic activity; **PEG** -5000-**catalase** retained 95%. **PEG**-5000-**catalase** resisted digestion by trypsin, chymotrypsin, and a protease from Streptomyces griseus. **PEG**-1900-**catalase** and **PEG**-5000-**catalase** exhibited enhanced circulating lives in the blood of acatalasemic mice during repetitive intravenous injections. No evidence was seen of an immune response to injections of the modified enzymes. Mice injected repetitively with **PEG**-5000-**catalase** remained immune competent for unmodieied **catalase** , and no evidence of tissue or organ damage was seen.